TECHNICAL NOTES

RELATIONSHIP BETWEEN AREAS UNDER ULTRACENTRIFUGAL SEDIMENTATION PATTERNS AND CONCENTRATION FOR MILK PROTEINS

Interpretation of ultracentrifugal results often requires estimation of concentrations from areas defined by sedimentation patterns. This is especially true in studies of unknown mixtures, such as may be produced by heating milk, and of known mixtures in which interactions may occur. Although proteins are basically similar in structure, and presumably similar in refractive index, there is need of assurance as to the limits within which constant proportionality between area and concentration may be assumed. Accordingly, many sedimentation patterns on known systems were accumulated and remeasured. Essential results are shown in Figures 1 and 3. The conclusion is that for all small molecules, and even for moderately large ones ($S = 100 \times 10^{-13}$), the concentration/area factor has a single definite value within narrow limits; but that above $S = 100 \times 10^{-13}$, approximately, the factor may be expected to increase with particle size. The breakdown in constant proportionality occurs at particle diameters of the order of 500Å, roughly coincident with onset of light scattering.

All patterns analyzed were obtained with the Spinco Model E ultracentrifuge,[1] most using the standard 12-mm. cell, but some using the synthetic boundary cell, and one (for ovalbumin) using a 1.5-mm. cell.

Only patterns for which a base line was clearly defined or was separately determined were included. Tracings on heavy 1-in.-1/10-

[1] Mention of trade names is for the purpose of identification only and does not imply endorsement of the product or its manufacturer by the U. S. Department of Agriculture.

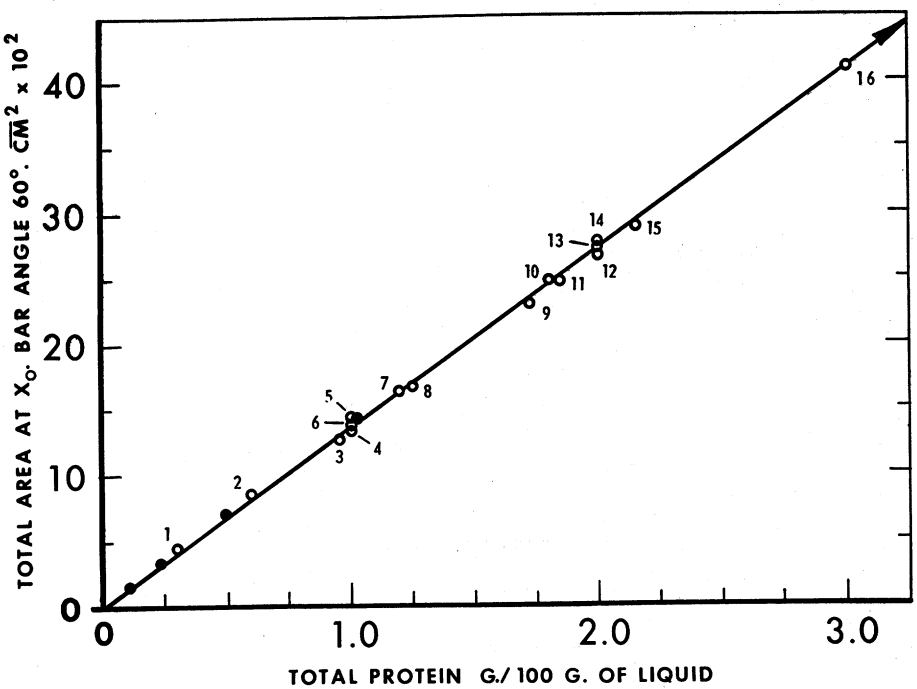

FIG. 1. Relationship of areas under Schlieren patterns to concentration for various proteins. The four solid circles are for bovine serum albumin in Veronal, pH 8.6. The numbered open circles represent: (1) snail blood, *Otala lactea*, $S = 89 \times 10^{-13}$; (2) α-casein, pH 11.2; (3) centrifuge milk serum, dialyzed against Veronal, pH 8.5, three components resolved; (4) α-casein complex fraction, washed in water and dialyzed against Veronal, pH 8.3, four components resolved; (5) centrifuge milk serum, dialyzed against Veronal, pH 8.5; (6) β-lactoglobulin, pH 6.8, two components unresolved; (7) α-casein, pH 12.2; (8) ovalbumin in 0.05 *M* NaCl; (9) small size fraction of the casein complex, washed in water, dialyzed against Veronal, pH 8.4, three components resolved; (10) medium-sized fraction of the casein complex, washed in water, dialyzed against Veronal, three components resolved; (11) large-sized fraction of the casein complex, washed in water, dialyzed against Veronal, three components resolved; (12) α-casein in Veronal; (13) bovine serum albumin plus γ-globulin, 1% each, pH 6.6; (14) large-sized fraction of the casein complex, washed, dialyzed against Veronal, three components resolved; (15) α-casein complex fraction treated with sodium tetrametaphosphate at pH 8.6; (16) α-lactalbumin plus β-lactoglobulin, 1.5% each, pH 6.9, two components resolved. The line through these various points is drawn with an arrow because the ovalbumin area (8) is actually calculated from the area for a 10% solution in a 1.5-mm. cell.

inch cross-ruled paper were prepared by use of an enlarger at a magnification of 6.39 cm. per centimeter in most cases. Either the left-hand reference line or the meniscus was set on a heavy (1-in.) vertical line, and the lower margin of the base on a heavy horizontal line. The lower margin of the complete pattern was then traced in red; next the paper was moved to set the upper margin of the base on the red tracing and the upper margin of the complete pattern then traced in blue. Finally, an average, black outline was drawn between the red and blue tracings. For this a fine ball-point pen is convenient, since it leaves a neat track for the planimeter needle.

Each planimeter tracing consisted of six complete circuits of the pattern; the instrument being read after the first and sixth circuits. The difference between these readings divided by five was taken as the area. Such measurements were always made at least in duplicate, usually several times. The average mean deviation of such average area measurements is 0.165×10^{-2} $\overline{cm}^2$ on the scale of Figures 1, 2, and 3, making the average error for small proteins 1% at a concentration of 1.2%, for example. The first circuit in a set of six is discarded, because it is found to be often in error, due to backlash and for other reasons.

In the various centrifugations bar angles as low as 30° and as high as 70° were used. All areas were corrected to a bar angle of 60° by multiplying by the factor tan (90°-60)/tan (90°-θ). No deviations in areas attributable to changes in bar angle were observed, even at concentrations as low as 0.1%.

All areas given in Figure 1 are at zero equivalent centrifuging times, i.e., at zero fall distance. Extrapolations were made by plotting the measured areas from successive frames of a set against fall distance ($X_{peak} - X_{meniscus}$), and by plotting the measured areas against $X_p^2/X_{men.}^2$. Both extrapolations tend to give the same areas at zero equivalent centrifuging time.

The solid lines of Figure 2 show three typical plots of areas as actually measured: the dashed lines are the areas multiplied by X_p^2/X_m^2, the theoretical correction for radial dilution. In only one case, for ovalbumin, is the theoretical correction adequate. Not only may the theoretically corrected plots slope either up or down

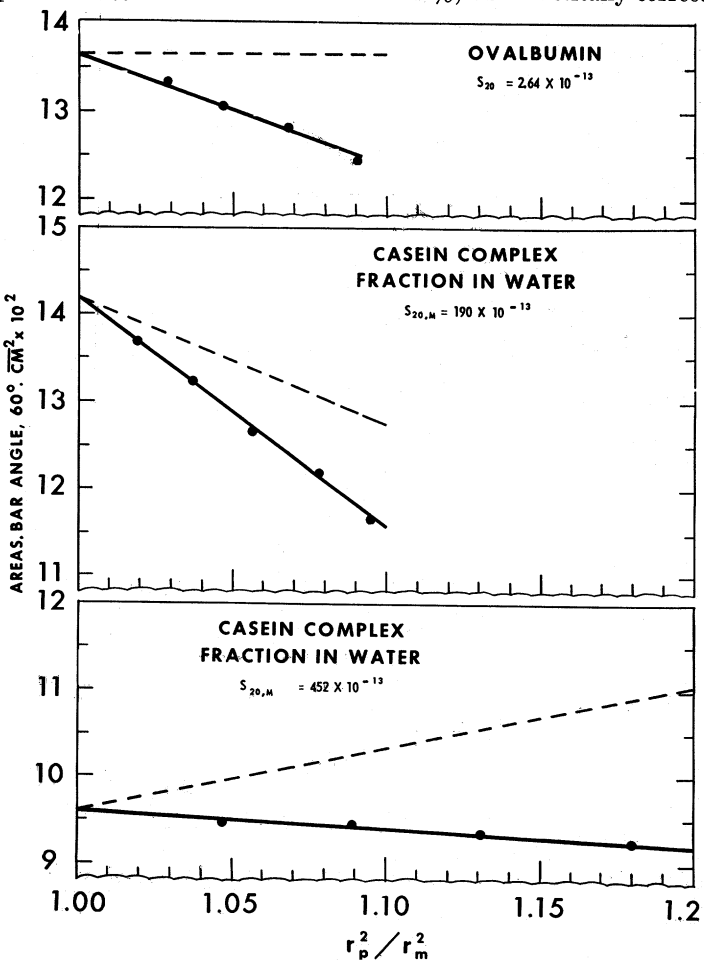

FIG. 2. Typical plots of measured areas against X_p^2/X_m^2, together with the areas corrected for theoretical radial dilution by this factor, showing the magnitude of errors possible by using corrected areas for single patterns.

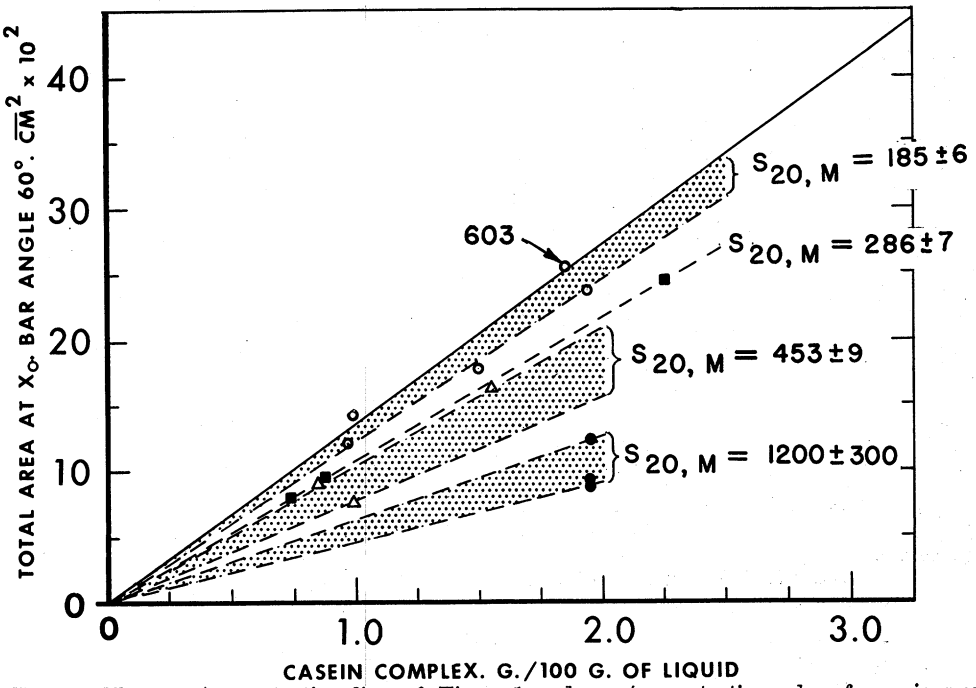

FIG. 3. The area/concentration line of Figure 1, and area/concentration values for various casein complex fractions prepared by centrifuging. These fractions were all washed with water, dispersed in water, and the dispersions all found to be at pH 7.5, approximately. The fractions represent narrow particle size ranges but are still polydisperse. The sedimentation constants given are the dominant values corresponding to the peaks of the Schlieren patterns. These sedimentation constants have been corrected to the viscosity and density of average milk serum, and to the average voluminosity of the casein colloid in milk. The values observed, in water, are 2 to 50% higher than the corrected values given.

but they are sometimes curved. These deviations may be attributed to polydispersity, asymmetry, gelation, etc. Detailed discussion will be presented elsewhere. For the present purpose, it is sufficient to note that correction of single patterns by the theoretical radial dilution factor may lead to large errors. Some sort of extrapolation to zero equivalent centrifuging time is essential.

Multiple peaks when resolvable should be plotted separately and the intercepts at $t = 0$ added together as a check on the total area. In cases where a pattern is clearly resolved, and symmetrical, but one leg is partly hidden, as occurs near the meniscus and again near the base of the cell, the truncated areas thus defined will be low. If these outlines be completed, however, by drawing in the mirror image of the leg which is definite or can be resolved, then the area of the new pattern will fall in line with the others.

Figure 3 is of especial interest with respect to the casein complex. It is noted first that the sedimentation constants 185, 286, and 453 correspond to particle volumes in the ratio 1:2:4, approximately. This follows from Stokes' law, $r = \sqrt{\frac{9}{2}\frac{\eta s}{(\rho_p - \rho_m)}}$. The departure of the indicated concentrations from the actual concentrations are roughly in the same ratios. This would be expected, since at a given concentration light scattering is theoretically proportional to particle volume. It will be noted also that for each average s value the deviations in areas are much greater than the deviations in s. For each of these fractions the voluminosity, the ratio of the total particle volume to the volume of contained protein, was calculated from viscosity measurements.[2] In general, for a given s value, the preparations having high voluminosities deviate least from the correct area/concentration line. In the one preparation designated by Experiment No. 603, the voluminosity found was 13, as compared with an average value of 4.3 for the colloid in normal milk. In this case, therefore, a loose structure is indicated, probably sufficiently transparent to light as to have the same effect in light bending as if it were a collection of small protein molecules.

T. F. FORD[3]
Dairy Products Laboratory
Eastern Utilization Research and Development Division, USDA
Washington, D. C.

[2] *cf.* Ford, T. F. Viscosity-Concentration and Fluidity-Concentration Relationships for Suspensions of Spherical Particles in Newtonian Liquids. J. Phys. Chem., 64: 1168. 1960.

[3] Present address: U. S. Naval Research Laboratory, Washington, D. C.